

**Frederick National Laboratory  
for Cancer Research**

*sponsored by the National Cancer Institute*

**Vaccine, Immunity and Cancer Directorate  
Standard Operating Procedure**

**SOP Title:** Use and Maintenance of Pipettes

**Document ID:** 26009

Version

1.0

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Supersedes

New

**Effective Date:** 29 Nov 21

**Written by:**

Printed Name:

Title:

Signature/Date:

**Approved by:**

Printed Name:

Title:

Signature/Date:

**QA Approved by:**

Printed Name:

Title:

Signature/Date:

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## 1. PURPOSE

- 1.1. The purpose of this procedure is to describe the use and maintenance of calibrated pipettes.

## 2. SCOPE

- 2.1. This procedure applies to all calibrated pipettes, manual and electronic single-channel pipettes and multi-channel Pipettes.
- 2.2. This procedure does not apply to pipettes that do not have routine calibration performed (For example, pipet aids/pipettors).

## 3. REFERENCES

- 3.1. Rainin Original Certificate of Conformity
- 3.2. Rainin Classic Continuously Adjustable Digital Microliter Pipette User Manual
- 3.3. 10007: Non-Routine Equipment Maintenance
- 3.4. 10009: General Record Review
- 3.5. 15000: Waste Disposal at the Advanced Technology Research Facility
- 3.6. 26012: Use and Maintenance of an Analytical & Precision Balance
- 3.7. 26016: Operation, Use and Maintenance of the Water Purification Systems

## 4. RESPONSIBILITIES

- 4.1. The Research Associate, hereafter referred to as Analyst, is responsible for reviewing and following this procedure, and documenting performance of equipment maintenance.
- 4.2. The Quality Control Analyst is responsible for reviewing and following this procedure. Quality Control Analyst is responsible for maintaining calibrations.
- 4.3. The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.
- 4.4. The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.
- 4.5. Trained personnel perform equipment maintenance record review per "10009: General Record Review."

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## 5. DEFINITIONS

- 5.1. As Needed Maintenance - Maintenance that is performed outside of routine maintenance but is not performed in response to equipment malfunction.
- 5.2. BioClean - Free or almost free from harmful microorganisms.
- 5.3. Non-Routine Maintenance - Maintenance that is performed in response to equipment malfunction or failure.
- 5.4. Routine Maintenance - Maintenance that is performed at planned intervals to identify and prevent problems before they result in equipment failure.
- 5.5. TOC – Total Organic Carbon
- 5.6. Type II Water - Water, Type II – Pure/Analytical Grade, used for standard applications (Resistivity >1 MΩ-cm and TOC ≤ 50 ppb).

## 6. REAGENTS, CHEMICALS, AND EQUIPMENT

- 6.1. Analytical Balance
- 6.2. Bleach, Clorox, Concentrated (FNLCR Warehouse, Cat # 68100251 or equivalent)
- 6.3. Pipettes
- 6.4. Pipette Stand or Magnetic Hang-ups
- 6.5. Pipette Tips
- 6.6. 50 mL Reagent Reservoir (FNLCR Warehouse, Cat # 66401270 or equivalent)
- 6.7. Ster-ahol (Fisher, Cat # 19-546-862 or equivalent)
- 6.8. 15 mL Tubes, Conical (FNLCR Warehouse, Cat # 66401479 or equivalent)
- 6.9. Water, Type II
- 6.10. Weigh Boats (VWR, Cat # 89106-768 or equivalent)
- 6.11. Weigh Paper (VWR, Cat # 12578-121 or equivalent)
- 6.12. Wipe, Low-Lint, Wypalls (FNLCR Warehouse, Cat # 79300335 or equivalent)

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## **7. HEALTH AND SAFETY CONSIDERATIONS**

- 7.1. Proper safety precautions must be taken while working in a laboratory setting. This includes, but is not limited to, proper protective equipment such as lab coats, safety glasses, closed-toe shoes, and non-latex gloves.
- 7.2. Refer to the respective Safety Data Sheet (SDS) when working with any chemicals.
- 7.3. Refer to “15000: Waste Disposal at the Advanced Technology Research Facility,” “EHS-WM-1: Disposal and Minimization of Chemical Waste,” and “EHS-WM-2: Biological Waste Handling and Disposal” for waste disposal processes.

## **8. PROCEDURE PRINCIPLES**

- 8.1. The Vaccine, Immunity and Cancer (VIC) Directorate currently uses Rainin brand pipettes for air displacement pipetting. Only use Rainin brand pipette tips with Rainin brand Pipettes.
- 8.2. Do not store pipettes horizontally.
- 8.3. Do not invert or lay the pipette flat with liquid in the pipette Tip.
- 8.4. Do not allow any liquid to enter the body of the pipette.
- 8.5. Pipette shaft and tip ejectors are autoclavable.
- 8.6. If a pipette is dropped or mishandled, it can disrupt the accuracy of the pipette. If a pipette is ever suspected to not be dispensing the correct volume, it is recommended to confirm the pipette's accuracy per section 11 along with recording the event as a non-routine maintenance (section 10.2).
- 8.7. Using the pipette consistently with a steady rhythm is key for greater accuracy and sample – to – sample reproducibility along with holding the pipette in a vertical position throughout the pipetting cycle.
- 8.8. See Attachment 1: Parts/components of pipette

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## 9. EQUIPMENT USE

### 9.1. Volume Setting

9.1.1. To eliminate errors due to mechanical backlash when setting the desired volume, first turn the Knob 1/3 turn above the desired volume. Then turn the Knob slowly clockwise until the desired volume is displayed.

9.1.2. Always dial down to the desired volume.

### 9.2. Pipette Tips

9.2.1. To mount a pipette tip, press the pipette shaft into the end of the pipette tip with light force. The pipette tip will seal properly on the pipette shaft with minimal force and positive pressure. Do not use more force than is required.

9.2.2. When using a multi-channel pipette, look at the tips to ensure each is properly seated.

### 9.3. Aspirating Liquid

9.3.1. Press plunger down to first stop.

9.3.2. When using a pipette, hold pipette vertical (a 90° angle to the surface of the liquid or within 20° of vertical), and immerse the pipette tip to the suggested depth (typically 2-6 mm below the meniscus) to prevent improper measurement. See Table 1.

Table 1: Suggested Immersion Depths for Various Pipettes

Model	Immersion Depth
2 - 10 µL	1 - 2 mm
20 - 100 µL	2 - 3 mm
200 - 1000 µL	3 - 6 mm
5000 µL, 10 mL	6 -10 mm

**Note:** The correct tip immersion depth can improve accuracy by up to 5%.

9.3.3. Allow plunger to return to up position slowly and wait approximately 1 – 3 seconds before slowly removing pipette tip from sample.

9.3.3.1. **Note:** Viscous material may require a slower plunger return and longer wait time.

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- 9.3.4. Some samples can leave residue within pipette tip after dispensing, such as serum and detergent, leading to inaccurate dispense volumes. Since this residue film remains constant in subsequent pipetting it is recommended to pre-wet pipette tip with sample then refilling pipette tip with sample before dispensing.
- 9.3.5. Slowly drag the side of the pipette tip along the inside the tube to remove any residual sample on the outside of the pipette tip.
- 9.3.6. If air bubbles are seen in pipette tip after aspiration, return sample to original container and repeat aspiration.
- 9.3.7. When using a multi-channel pipette, look at the pipette tips to ensure each tip has an equal volume of sample.

#### 9.4. Dispensing Liquid

- 9.4.1. Hold pipette tip at an angle along the inside wall of the tube or plate well to use capillary action which helps draw sample out of pipette tip.

**Note:** This technique can increase accuracy by 1% or more.

- 9.4.1.1. Dispensing into the center of a tube or well may result in residual volume remaining in pipette tip and insufficient volume transfer.
- 9.4.2. Use slow and consistent plunger pressure and speed to the first stop, then slowly continue to the second stop to blowout the sample.

**Note:** During blow out phase, slowly lift pipette tip above sample to avoid bubbles.
- 9.4.3. Do not allow plunger to snap back to the up position.
- 9.4.4. After dispensing sample volume, look at the pipette tip(s) to ensure appropriate volume has been transferred.

#### 9.5. Eject Pipette Tip

- 9.5.1. Use tip ejector button to eject pipette tip.

#### 9.6. Ergonomics

- 9.6.1. During consistent repetitive pipette tasks within a day, see Attachment 2: Examples of Stretches for Use While Pipetting.

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## 10. MAINTENANCE

### 10.1. Semi-Annual Calibration

**Note:** All Rainin pipettes come with a certificate of conformity, which indicates a date that the pipette was tested prior to shipping, to show the pipette met with all pipette specifications. If a pipette cannot be calibrated immediately on receipt; the certificate of conformity can be used in place of a pipette calibration for up to 6 months after the date of receipt.

10.1.1. Pipettes are calibrated by a qualified vendor every 6 months, or if damage to the pipette occurred.

10.1.2. The vendor performs an “As Found” and “As Left” check on each pipette and perform the calibration at a minimum of the 10% and 100% volume settings, which will change for each Pipette based on the Pipette volume.

### 10.2. Non-routine maintenance

10.2.1. In the case that the pipette is not operating correctly, transition processes being performed to another unit (when applicable), post a sign stating the equipment is out of service and initiate non-routine maintenance documentation per “10007: Non-Routine Equipment Maintenance.”

10.2.2. Document the nature of any failures or malfunctions, how and when it was discovered, and the personnel involved on “10007-01: Non-Routine Equipment Maintenance Form.”

## 11. CONFIRMATION OF PIPETTE ACCURACY

**Note:** The density of water is 1 g/mL. This means that every microliter ( $\mu\text{L}$ ) should weigh 0.001 g (or 1 mg).

**Note:** Confirmation of a pipette accuracy occurs only if suspected damage, drop, pipette performance issues, or a vendor cannot certify the pipette prior to the semi-annual calibration due date.

11.1. Perform calibration check on the Analytical Balance, per “26012: Use and Maintenance of an Analytical & Precision Balance.”

11.2. Fill a 15 mL conical tube with type II water and allow water to equalize to room temperature next to the analytical balance and the pipette being tested.

**Note:** Let type II water equalize to room temperature for at least 30 minutes.

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11.3. The pipette is to be checked at two settings, at the low and high point of the pipette's range.

11.3.1. For example, for a 20-200 µL pipette: 20 µL and 200 µL

11.4. Place weigh boat or weigh paper onto the deck of the balance and press «→0/T←» to tare/zero the balance.

11.5. **Note:** Weigh paper can be used for volumes under 20 µL. Otherwise, a weigh boat is used.

11.6. Set the pipette to volume being checked and carefully aspirate water into pipette Tip. Dispense the water onto weigh boat or weigh paper. Measure weight of water to one decimal place.

11.7. Wait for the weight to stabilize then print and/or record reading.

11.8. Repeat steps 11.1.4 to 11.1.7 nine more times, for a total of ten weights at a single volume.

11.9. Repeat the steps 11.1.4 to 11.1.7 for other volumes being checked.

11.10. Average the results for each volume together using the following equation:

$$\text{Average} = \frac{\text{Volume 1} + \text{Volume 2} + \text{Volume 3} + \dots}{10}$$

**Note:** For example, 20-200 µL pipette: 20 µL and 200 µL  
Average the volumes recorded for the 20 µL volume and separately average the volumes recorded for the 200 µL volume.

11.11. Determine the accuracy using the following equation:

$$\text{Accuracy} = \frac{|\text{Measured Average} - \text{Actual Volume}|}{\text{Actual Volume}} \times 100$$

**Note:** For example, 20-200 µL pipette: 20 µL and 200 µL  
Calculate the accuracy for the 20 µL volume, and separately calculate the accuracy for the 200 µL volume.

11.12. The accuracy for a pipette should be less than 2% for each volume measured to be considered acceptable. If a pipette does not meet this requirement, the check should be repeated by another analyst.

11.13. If the pipette fails a second time, remove it from service and give to Scientific Manager or designee to handle for further actions required.

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- 11.14. Record calibration check in 26009-01: Pipette Accuracy Confirmation Form, include calculations and balance print outs.

**12. ATTACHMENTS**

- 12.1. Attachment 1: Parts/components of Pipette
- 12.2. Attachment 2: Examples of Stretches for Use While Pipetting
- 12.3. Attachment 3: 26009-01: Pipette Accuracy Confirmation Form

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### 13. REVISION HISTORY

Version #	Changes	Reasons
1.0	<ol style="list-style-type: none"><li>1. SOP renumbered from HSL_EQ_012.</li><li>2. Update Language</li><li>3. Added additional guidance for Use and Maintenance</li><li>4. Added pipette verification process and form</li><li>5. Added Non-routine maintenance section</li><li>6. Updated SOP nomenclature</li></ol>	<ol style="list-style-type: none"><li>1. Reflect numbering scheme per SOP 10000.</li><li>2. Clarity</li><li>3. GDP compliance</li><li>4. Reflect new practice</li><li>5. Reflect GCLP guidance</li><li>6. Reflect new practice</li></ol>

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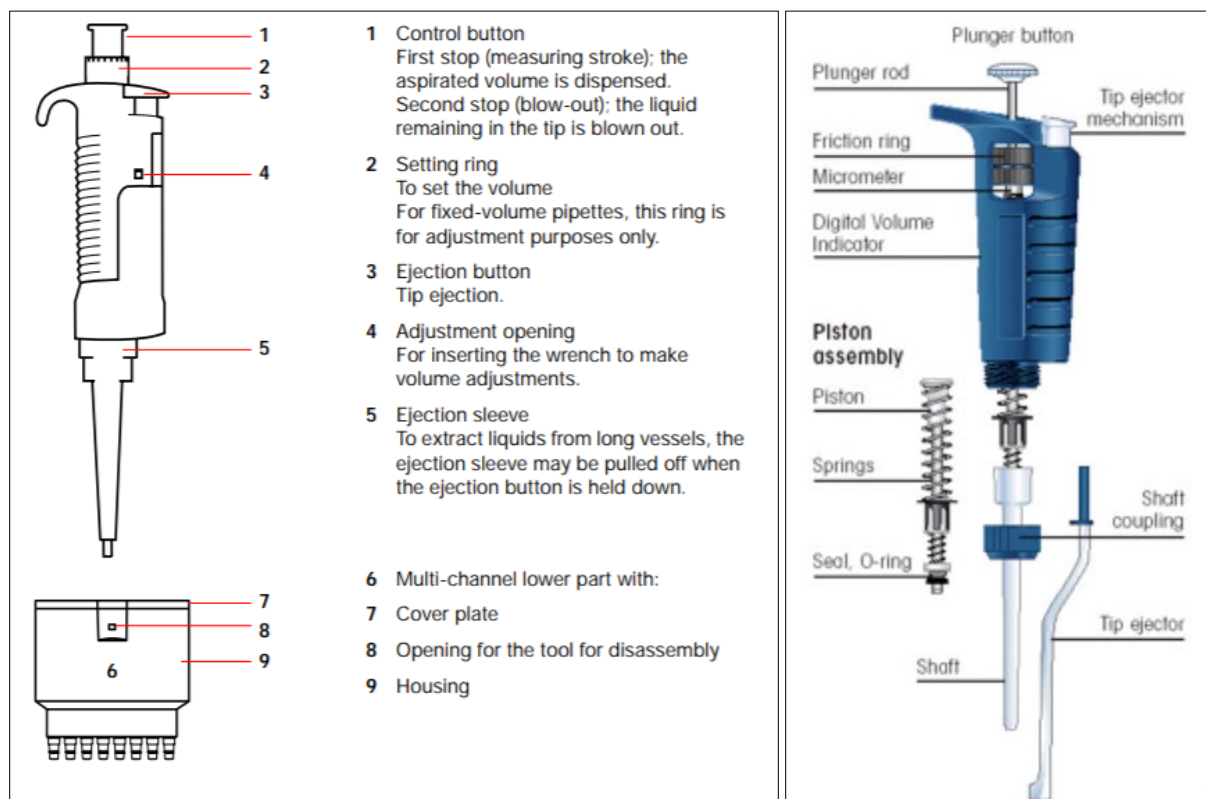
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### Attachment 1: Parts/components of Pipette



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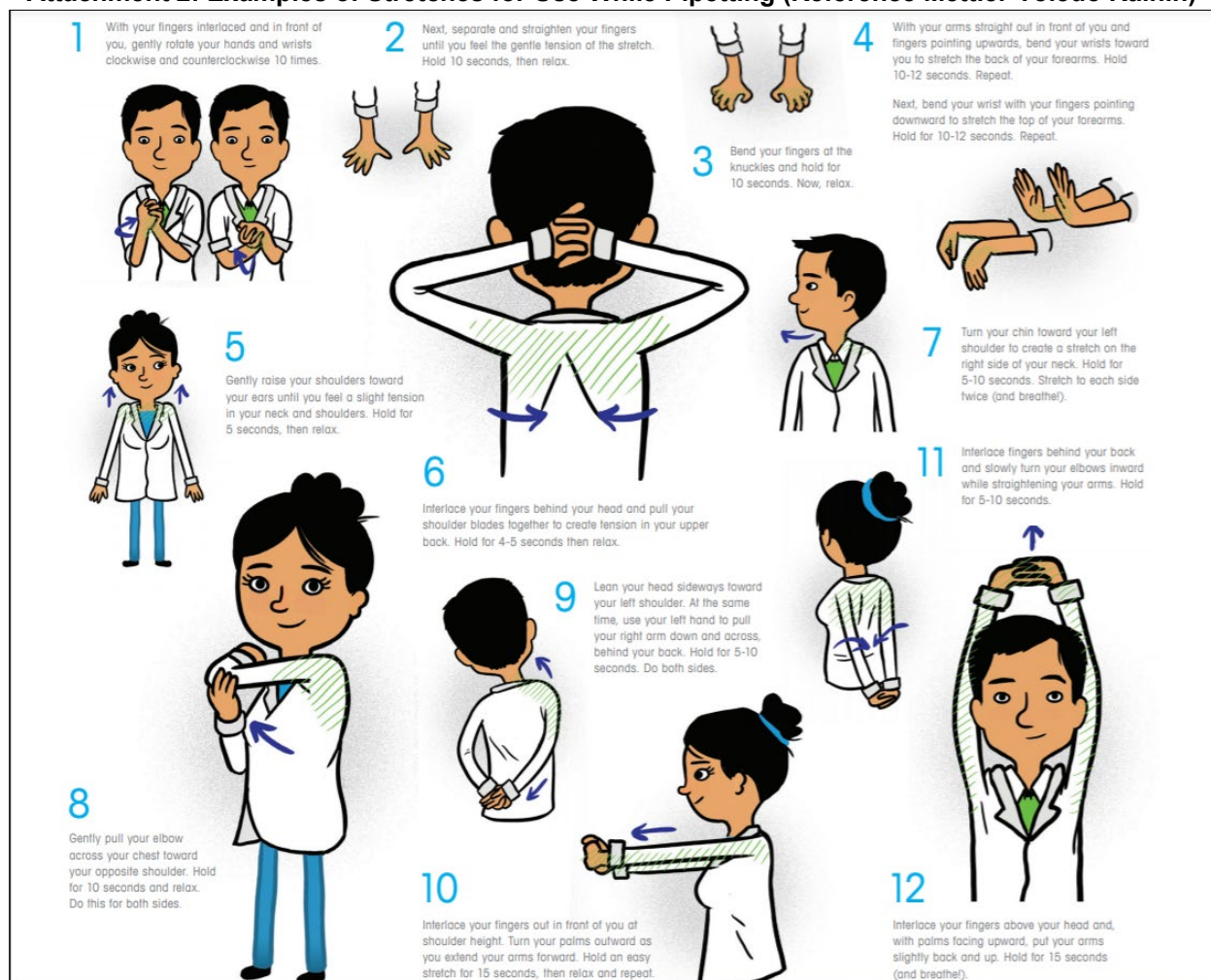
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**Attachment 2: Examples of Stretches for Use While Pipetting (Reference Mettler-Toledo Rainin)**



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### Attachment 3: 26009-01: Pipette Accuracy Confirmation Form

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<b>Form Title:</b> Pipette Accuracy Confirmation Form			
<b>Document ID:</b> 26009-01		<b>Version:</b>	1.0
Associated SOP: 26009		<b>Effective Date:</b>	29 Nov 21
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#### Equipment

Equipment Name	Equipment ID	Calibration Due Date
Pipette: $\mu\text{L}$	PIP_	
Water System <input type="checkbox"/> NA <input type="checkbox"/> HSL_ <input type="checkbox"/> Other:		
Analytical Balance <input type="checkbox"/> HSL_ <input type="checkbox"/> Other:		
Weight Set <input type="checkbox"/> HSL_ <input type="checkbox"/> Other:		

#### Results

Measurement	Volume 1: $\mu\text{L}$	Volume 2: $\mu\text{L}$
Replicate Number	Mass (mg)	Mass (mg)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
Average		
Expected (1 $\mu\text{L}$ = 1 mg)		
Accuracy ( $< 2\%$ )		
Results	<input type="checkbox"/> Pass <input type="checkbox"/> Fail	<input type="checkbox"/> Pass <input type="checkbox"/> Fail
Performed By/Date:		

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☐ N/A Second Run Results

Measurement	Volume 1:	$\mu\text{L}$	Volume 2:	$\mu\text{L}$
Replicate Number	Mass (mg)		Mass (mg)	
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
Average				
Expected (1 $\mu\text{L}$ = 1 mg)				
Accuracy ( $< 2\%$ )				
Results	<input type="checkbox"/> Pass <input type="checkbox"/> Fail		<input type="checkbox"/> Pass <input type="checkbox"/> Fail	
Performed By/Date:				

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#### Attachments

#### Comments

- ☐ First run pass.
- ☐ First run fail, second run pass.
- ☐ First run fail, second run fail. Discuss with Scientific Manager.

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